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MANUAL

FOR THE

PHYSIOLOGICAL CHEMICAL LABORATORY

BY

JOHN A. MANDEL

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MANUAL

FOR THE

PHYSIOLOGICAL CHEMICAL LABORATORY.

FOR THE USE OF MEDICAL STUDENTS.

BY

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NEW YORK.
JOS. V. STANDISH.
1897.

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Manual for the Physiological Chemical Laboratory.

Lesson I.

CARBOHYDRATES.

A compound containing C, H, and O, where the H and O are in the proportion to form water.

Three groups.

MONOSACCHARIDES. $C_6H_{12}O_6$.	DISACCHARIDES. $C_{12}H_{22}O_{11}$.	POLYSACCHARIDES. $C_6H_{10}O_5$.
Dextrose, glucose,	Cane sugar,	Starch,
Lævulose,	Lactose,	Dextrin,
Inosite,	Maltose,	Glycogen,
Galactose,		Cellulose.

DEXTROSE, GLUCOSE, GRAPE, SUGAR, ETC.

The most important member of the first group is dextrose, which occurs in certain fruits and grains, and in the blood, lymph, chyle, diabetic urine, etc.

Experiment 1. Place some in a test tube with water. It dissolves. It has a sweetish taste.

Experiment 2. Fill a test tube one quarter full with silver nitrate ($AgNO_3$) solution, and add a few drops

of ammonium hydrate (NH_4OH) until the precipitate formed just dissolves. Add a few drops of the above dextrose solution to this, and warm in water-bath. A deposit of a metallic silver mirror is obtained, showing that dextrose has a reducing action on certain metallic solutions when in an alkaline condition, and most of the chemical tests for dextrose depend on this property.

Experiment 3. Trommer's test. Place a few drops copper sulphate ($CuSO_4$) in a test tube, and an equal amount of water, and then caustic soda (NaOH) until a little of the copper hydrate formed remains undissolved. Add to this a drop or two of the dextrose solution. The copper hydrate dissolves. Apply gentle heat. A yellow reduction of hydrated sub-oxide of copper, and then red sub-oxide of copper (Cu_2O), separates even below the boiling point.

Care must be taken not to have an excess of either copper sulphate or caustic soda in this test, otherwise interferences are liable to occur.

Experiment 4. Moore's test. Make some of the dextrose solution alkaline with caustic soda, and apply heat. The solution turns yellow, red, brown, or black, depending upon the amount of dextrose in the solution. Add a few drops of nitric acid (HNO_3) to the solution. The color disappears, and an odor of caramel or burnt sugar is given off.

Experiment 5. Fehling's test. Place some copper sulphate in a test tube, and an equal amount of caustic soda solution, then a solution of Rochelle salt (sodium-potassium tartrate $NaKC_4H_4O_6$, $4H_2O$) until the precipitate is dissolved, and a clear, deep blue solution is

obtained. This constitutes Fehling's solution. Heat the upper part of the liquid to the boiling point by holding the test tube at the lower end and applying the heat above. No change should occur. Add a drop of the dextrose solution. Immediate reduction of the yellow hydrated sub-oxide of copper, and then a red sub-oxide of copper.

Experiment 6. Barfoed's test. Add some of Barfoed's solution (a solution of copper acetate with acetic acid) to some of the dextrose solution in a test tube, and apply heat. A reduction of copper sub-oxide is produced. This test distinguishes dextrose from lactose and maltose.

Experiment 7. Boettger's test. Fill a test tube one half full of water, add a little of the dextrose solution and some solid bismuth sub-nitrate, and now make alkaline with sodium carbonate (Na_2CO_3). Heat to boiling. The white bismuth sub-nitrate will turn gray, brown, or black, depending on the amount of dextrose in the solution, and will collect at the bottom of the test tube.

Experiment 8. Place some of the dextrose solution in the saccharometer, and add a small piece of yeast thereto. Place in a warm locality at a temperature of about 40° C. Bubbles of gas collect after a time. This is called fermentation, and the gas which collects is carbon dioxide (CO_2), while another body, alcohol (C_2H_5OH), remains in the liquid. $C_6H_{12}O_6 = 2\,C_2\text{-}H_5OH + 2\,CO_2$.

CANE SUGAR.

Occurs in the juices of many plants, in fruits, in flowers, and in honey; does not occur in the animal

body, but undergoes transformations when partaken of.

Experiment 9. Place some cane sugar in some water in a test tube. It dissolves readily, forming a solution having a sweetish taste.

Experiment 10. Repeat Experiment 5, using a solution of cane sugar instead of dextrose. No reduction on the application of heat.

Experiment 11. Fill a test tube one half full with the cane sugar solution, acidify with a drop or two of sulphuric acid (H_2SO_4), and boil for a few minutes. This operation will convert the cane sugar into a mixture of dextrose and lævulose, called *invert sugar*, $C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6$.

This process is similar to the action of the invertin of the intestinal juice on cane sugar in the intestin.

Experiment 12. Neutralize this solution with caustic soda (NaOH), and repeat Experiment 5. A reduction of the red sub-oxide is obtained, showing the presence of dextrose and lævulose.

Cane sugar does not ferment under the action of yeast, but readily undergoes lactic acid fermentation under the action of certain ferments.

LACTOSE OR MILK SUGAR.

Important constituent of the milk of mammals and is sometimes found in the urine of women in the early days of lactation, or after weaning.

Experiment 13. Place some of the lactose in water in a test tube. It does not dissolve very readily and has a feeble sweet taste.

Experiment 14. Repeat Experiment 5, using a solu-

tion of lactose. A reduction similar to that obtained with dextrose is the result.

Experiment 15. Add some Kephir ferment to some lactose solution and keep warm for a short time, the solution turns acid as shown by placing a drop of the solution on a piece of blue litmus paper. This acid is lactic acid $C_{12}H_{22}O_{11} + H_2O = 4\,C_3H_6O_3$..

Lactose. Lactic acid.

MALTOSE OR MALT SUGAR.

Formed by the action of malt diastase on starch. Is the chief product of the action of saliva and pancreatic juice on starch during digestion.

Experiment 16. Dissolve some in water and observe the sweet taste of the solution.

Experiment 17. Repeat Experiment 5, using the above solution of maltose. A reduction is obtained but not so pronounced as with dextrose.

Experiment 18. Apply Barfoed's test (see Experiment 6) to the maltose solution. No reduction.

It really undergoes fermentation by yeast, and when heated with very dilute sulphuric acid it yields dextrose. Diastatic enzymes have a similar action.

STARCH.

Found in nearly all plants. Is most abundant in the cereals. Occurs as microscopic granules of various sizes and structure. Does not occur in the animal body except as food.

Experiment 19. Place some in water. Does not dissolve but disintegrates yielding a white mud.

Experiment 20. Boil some water in a test tube and

add some of the above mud thereto. An opalescent solution or jelly-like mass is the result, depending upon the amount of starch added.

Experiment 21. Place a little starch solution in a test tube and dilute with water. Add a drop of tincture of iodine thereto. Deep blue coloration is obtained, which disappears on heating and reappears on cooling again.

Experiment 22. Boil some starch solution with some dilute hydrochloric acid (HCl) for some time, neutralize with caustic soda (NaOH) and repeat Experiment 5, using a portion.

A reduction is obtained showing the presence of dextrose. The action of the acid is expressed by the following equation:

$$\underset{\text{Starch.}}{3C_6H_{10}O_5} + H_2O = \underset{\text{Dextrose.}}{C_6H_{12}O_6} + \underset{\text{Achroo-dextrin.}}{C_6H_{10}O_5} + \underset{\text{Erythro-dextrin.}}{C_6H_{10}O_5}$$

Experiment 23. Moderately heat some of the starch solution with an infusion of malt (containing diastase) for some time. It first becomes clear due to the formation of soluble starch. Apply Fehling's test (Experiment 5) to the product. A reduction is obtained due to the presence of maltose. The change is expressed by the following equation:

$$10\,\underset{\text{Starch.}}{C_6H_{10}O_5} + 4\,H_2O = 4\,\underset{\text{Maltose.}}{C_{12}H_{22}O_{11}} + 2\,\underset{\text{Dextrin.}}{C_6H_{10}O_5}.$$

The action of the ptyalin of the saliva and amylopsin of the pancreatic juice on starch is also represented by the above change.

GLYCOGEN OR LIVER STARCH.

Occurs chiefly in the liver, muscles, and nearly all tissues of the animal body, and is a regular constituent of all developing animal cells.

Experiment 24. Place a little in some water in a test tube. It dissolves yielding an opalescent solution having no marked taste.

Experiment 25. Add a drop of tincture of iodine to a portion of the above solution. A wine red coloration is obtained, which disappears on heating.

Experiment 26. Boil the other portion of the glycogen solution with some dilute hydrochloric acid (HCl), neutralize with caustic soda, and then test for dextrose by repeating Experiment 5. The boiling with the acid has converted the glycogen into dextrose and dextrin, similar to Experiment 22. Certain ferments such as that of the liver change glycogen into dextrose and dextrin.

DEXTRINS.

Found in the blood and muscles of animals to a slight extent. They are products of the diastatic ferments on starch, etc. Two varieties—Achroo-dextrin and Erythro-dextrin. Common dextrin is chiefly Erythro-dextrin.

Experiment 27. Place some in water in a test tube. It dissolves and a strong solution forms a gummy and sticky mass.

Experiment 28. Add some tincture of iodine to the dextrin solution. Red coloration.

Experiment 29. Boil some of the dextrin solution with dilute hydrochloric acid (HCl) for a few minutes. Neutralize with caustic soda (NaOH) and test for dextrose by Fehling's solution (Experiment 5). The acid has converted the dextrin into dextrose as shown by the reduction.

Lesson II.

FATS.

Almost all fats and oils are compound ethers of glyceryl, $C_3 H_5''$, or triglycerides.

The most important fats are tri-stearine $C_3 H_5 (C_{18} H_{35} O_2)_3$, tri-palmitine $C_3 H_5 (C_{16} H_{31} O_2)_3$, and tri-olein $C_3 H_5 (C_{18} H_{33} O_2)_3$. These are compound ethers of stearic, palmitic, and oleic acids with glyceryl.

Experiment 1. Place some of the fat in water in a test tube. It does not dissolve but floats on the liquid, hence it is lighter than water.

Experiment 2. Apply heat to the water and observe that the fat melts much before the water begins to boil.

Experiment 3. Rub a little of the fat on a piece of paper and observe that it makes a transparent spot.

Experiment 4. Heat a little of the fat on foil with some potassium bisulphate ($KHSO_4$) and note that it melts readily, flowing about the foil and giving off a marked and characteristic irritating odor of *acrolein.*

It inflames, burning with a sooty flame.

Experiment 5. Shake some of the liquid fat given with water in a test tube. The oil globules readily conglomerate and rise to the surface.

Experiment 6. Shake another portion with an albumin solution. The oil globules do not readily conglomerate, but form a milky white emulsion.

Experiment 7. Shake a third portion of the liquid fat with water containing a little soap solution. An immediate and permanent emulsion is obtained on slightly shaking.

These facts are of the greatest importance in the digestion and assimilation of fats in the intestinal tract.

Experiment 8. Add an alcoholic solution of caustic soda to some of the solid fat and heat on the water-bath for some time. The product, a soap, is characterized by its forming a lather if shaken with water. This process is called saponification, and may be expressed by the following equation:

$$\underset{\text{Tri-stearin.}}{C_3H_5(C_{18}H_{35}O_2)_3} + \underset{\text{Caustic soda.}}{3NaOH} = \underset{\text{Sod. stearate or soap.}}{3NaC_{18}H_{35}O_2} + \underset{\text{Glycerin.}}{C_3H_8O_3}.$$

Experiment 9. Treat some of the soap solution with some dilute sulphuric acid (H_2SO_4). A decomposition with the precipitation of the insoluble fatty acids is the result.

$$\underset{\text{Soap.}}{2\,NaC_{18}H_{35}O_2} + \underset{\text{Sulphuric acid.}}{H_2SO_4} = \underset{\text{Stearic acid.}}{2C_{18}H_{36}O_2} + \underset{\text{Sod. sulphate.}}{Na_2SO_4}.$$

Experiment 10. Treat a small quantity of the liquid fat with some pancreatin and add a small amount of sodium carbonate (Na_2CO_3). Heat in the water-bath at 40° C. for some time. The fat-splitting ferment of the pancreas will decompose a part of the fat into fatty

acids and glycerine which combine with the sodium carbonate forming soaps which in turn cause the unacted on fats to form an emulsion on shaking.

$$\underset{\text{Tri-stearin}}{C_3H_5(C_{18}H_{35}O_2)_3} + 3H_2O = 3\underset{\text{Stearic acid.}}{C_{18}H_{36}O_2} + \underset{\text{Glycerin.}}{C_3H_8O_3}.$$

Experiment 11. Shake some water containing a little oil with ether. The ether dissolves the fat readily. Decant the ethereal solution into a watch glass, and allow the ether to evaporate, leaving the fat.

Experiment 12. Apply a little of the fat to a piece of blue litmus paper. No reaction.

Experiment 13. Apply a little of the rancid fat to a piece of blue litmus paper. A marked acid reaction is obtained, showing that the rancidity of fats is due to free fatty acids.

The free fatty acids form soaps directly, without decomposition, with sodium carbonate (Na_2CO_3) with the disengagement of carbon dioxide (CO_2) gas. They do not evolve an odor of acrolein when heated with potassium bisulphate. The fatty acids have an acid reaction. These properties distinguish them from the neutral fats.

Lesson III.

PROTEIN SUBSTANCES.

The most important class of substances that occur in animal or vegetable organisms and necessary for the phenomena of life. They are highly complex in their constitution and contain C,H,O,N,S, and some P. The nitrogen is the most important element.

The protein substances are divided into various groups having different properties and characteristics. They are as follows: albumins, globulins, nucleo-albumins, albuminates, albumoses and peptones, coagulated proteids, hæmoglobin, glyco-proteids, nucleo-proteids, keratin, elastin, collagen, etc.

Experiment 1. Place a little of the albumin on foil and apply heat. It blackens, burns with a characteristic odor of burnt hair or horn, and leaves no residue. This odor is characteristic of nitrogenous organic compounds.

Experiment 2. Place some in water in a test tube. It dissolves, but not readily, yielding an opalescent solution.

Experiment 3. Apply heat to some albumin solution in a test tube. The solution becomes cloudy and finally a coagulum forms. This is called heat coagulation. Add a drop of acetic acid to the above and the coagulum will be increased.

Experiment 4. Saturate some albumin solution with ammonium sulphate $(NH_4)_2SO_4$. A precipitate of albumin is produced. All protein substances with the exception of peptones are precipitated by saturation with this salt. This is a means of separating peptones from other protein substances.

Experiment 5. Place a solution of albumin containing some sodium chloride (NaCl) in a parchment dialyser as described, and place it in a vessel of water. It will be found that the water outside will acquire a salty taste after a time, because the salt has passed through the membrane. The presence of the salt may be tested by placing some of the water in a test tube and adding some silver nitrate ($AgNO_3$), which will give a white precipitate. No albumin will be found in the water on the outside.

This process is called dialysis or diffusion through membranes. The salts are diffusible and are called *crystalloid* substances, while the protein bodies, with the exception of peptones, do not, or with difficulty, diffuse, and are called *colloid* substances. Dialysis is made use of in separating colloid from crystalloid bodies.

Experiment 6. Color reactions. Add some Millon's reagent to a little of the albumin solution. A precipitate is formed, which slowly at the ordinary temperature, but quickly at the boiling point, turns red, depending upon the amount of albumin present.

The solid proteids give the same reaction.

Millon's reagent consists of a solution of mercuric nitrate in nitric acid containing some nitrous acid.

Experiment 7. Xantho-proteic reaction. Add a drop

of nitric acid (HNO_3) to some solid albumin on a watch glass. It turns yellow and orange-yellow. After a little while add a few drops of ammonium hydrate (NH_4OH) or caustic soda (NaOH) when the spot becomes red.

Experiment 8. Biuret reaction. Place one or two drops dilute copper sulphate ($CuSO_4$) in a test tube and add an excess of caustic soda (NaOH). Add some albumin solution to this and apply heat. A violet color is produced which deepens in tint on boiling. A reduction of copper sub-oxide may take place.

Experiment 9. Add two or three drops nitric acid (HNO_3) to some dilute albumin solution. A precipitate occurs which re-dissolves on adding an excess of the acid.

Experiment 10. Place some nitric acid (HNO_3) in a test tube and allow the filtered albumin solution to flow down gently on its surface. A well-defined ring or zone of precipitated albumin appears at the juncture of the two liquids. Most mineral acids act in the same manner.

Experiment 11. Add some mercuric chloride ($HgCl_2$) to some albumin solution. A precipitate of albumin is obtained. Most metallic solutions have the same action, hence the use of albumin as an antidote in cases of metallic poisoning.

Experiment 12. Acidify some albumin solution with acetic acid ($C_2H_4O_2$) and add a few drops of potassium ferrocyanide ($K_4Fe(CN)_6$) solution. A white precipitate of albumin is obtained.

Experiment 13. Acidify some albumin solution with acetic acid and add a few drops picric acid

($C_6H_2(NO)_3OH$) solution. A white precipitate of albumin is produced.

Experiment 14. Add some alcohol to some albumin solution in a test tube. The albumin will be precipitated.

Experiment 15. Place a little dilute albumin solution in a test tube, add some pepsin and *one* drop hydrochloric acid, and place in the water bath at about 40° C. for fifteen minutes.

The pepsin will convert the albumin into albumoses and peptones, which are the chief products of the proteolytic ferments, such as the pepsin of the gastric juice and the trypsin of the pancreatic juice.

Acidify the solution with a few drops of acetic acid and heat to the boiling point. This precipitates the unconverted albumin, which may be removed by filtration and the presence of albumoses and peptones determined by applying the Biuret test. (See Experiment 8, which gives a rose-red color.)

Experiment 16. Repeat Experiment 15, but instead of using pepsin use pancreatin and show that the products are about the same in both cases.

In this experiment do not make the solution acid with hydrochloric acid, but add one drop of sodium carbonate solution instead.

Experiment 17. Add some peptone to some water in a test tube. It dissolves readily.

Experiment 18. Apply heat to a portion of this solution. No coagulation showing a difference from other protein substances.

Experiment 19. Add some nitric acid to some of the peptone solution. No precipitation.

Experiment 20. Apply the Biuret test to some peptone solution. A rose-red color is obtained instead of a violet color, as given by other protein substances.

Experiment 21. Place a peptone solution in a parchment dialyser and place it in a vessel of pure water. After a time the peptones will have diffused through the membrane, as shown by applying the Biuret test to the water on the outside.

Lesson IV.

DIGESTION.

Is to separate those constituents of the food which serve as the nutriment of the body from those which constitute the waste and to separate each in such a form that it may be easily taken up by the blood from the alimentary canal.

SALIVARY DIGESTION.

Experiment 1. Collect as much saliva in a test tube as possible.

Experiment 2. Apply a little to a piece of red litmus paper. It turns blue, showing it to be alkaline. Directly after a meal it may be acid.

Experiment 3. Add a drop of hydrochloric acid (HCl) to some saliva in a test tube and then a few drops of ferric chloride (Fe_2CL_6). A blood-red coloration denotes the presence of sulphocyanides. The depth of color varies with different saliva, because the amount of sulphocyanides varies very markedly.

Experiment 4. Make some dilute starch solution (as described in Lesson I., Experiment 20) and add thereto some fresh saliva and keep in the water-bath at a temperature of 50°–55° C. for some little time. Observe that the starch solution becomes clear. Test a portion of the solution with tincture of iodine when,

instead of a blue reaction for starch, a red coloration due to dextrin will be obtained.

Test the presence of sugar (maltose) in the liquid by means of Fehling's or Boettger's test. (See Lesson I.)

GASTRIC DIGESTION.

Experiment 5. Test the reaction of the artificial gastric juice given by means of a piece of blue litmus paper. It is found to be acid.

Experiment 6. Add a drop of silver nitrate to a little of the gastric juice. A white precipitate of silver chloride (AgCl) shows the presence of hydrochloric acid (HCl). This precipitate is soluble in ammonium hydrate (NH_4OH).

Experiment 7. Place a drop of the gastric juice on a piece of Congo red paper. It turns blue, due to the free hydrochloric acid.

Experiment 8. Spread a few drops of *Gunsburg's solution* (consisting of an alcoholic solution of phloroglucin-vanillin) out in a thin layer upon a watch glass, or porcelain dish, and then gently warm; then a drop of the gastric juice is allowed to flow across it, or a glass rod dipped in the solution is drawn across the plate. If free hydrochloric acid be present, a deep scarlet-red streak is developed.

Experiment 9. Test for lactic acid. Add a few drops of the gastric juice to some Uffelmann's solution. This solution is made by mixing a few drops neutral ferric chloride (Fe_2Cl_6) solution with 2 drops of pure carbolic acid (C_6H_5OH), and adding water until the solution has a beautiful amethyst-blue color.

In the presence of lactic acid the blue color is changed to yellow instantly.

Experiment 10. Place a piece of hard-boiled egg in a test tube and add a little of the gastric juice. Keep this at a temperature of about 40° C., and observe the swelling up of the edges, the corrosion and the gradual disappearance or solution of the albumin being converted into albumoses and peptones.

Experiment 11. Test the presence of peptones in the solution by means of the Biuret test. (See Lesson 3, Experiment 20.)

Experiment 12. Warm a little milk to about 40° C. and then add some of the neutral gastric juice. An immediate coagulation of the milk is observed, due to the enzyme called Rennin in the gastric juice.

PANCREATIC DIGESTION.

Experiment 13. Apply some of the pancreatic juice to a piece of red litmus paper. It turns it blue, showing it to be alkaline.

Experiment 14. Make some dilute starch solution and add some pancreatic juice thereto, and keep at a temperature of about 40° C. for some little time. Observe that the starch solution becomes clear and the presence of sugar (maltose) may be shown by applying Fehling's test to the solution.

The amylopsin of the pancreatic juice converts starch, etc., into maltose and dextrin.

Experiment 15. Place some liquid fat with some water in a test tube and add a little pancreatic juice; keep at a temperature of 40° C. for some time. A

perfect emulsion of the fat will be obtained if the contents of the tube is shaken. (See Experiment 10, Lesson 2.)

Experiment 16. Place a piece of hard-boiled egg in a test tube and add some of the pancreatic juice and keep at 40° C. for some time. Observe the action on the albumin and the gradual solution of the piece.

The trypsin converts the albumin into albumoses and peptones which may be tested for by the Biuret test. (See Lesson 3, Experiment 20.)

Lesson V.

BLOOD AND BILE.

BLOOD.

Observe the obtainment of salt plasma from the blood of a dog.

Also the collection of blood in a vessel surrounded with an ice mixture.

Observe the coagulation of the salt plasma on the addition of water.

Also the coagulation of the cold blood on allowing it to attain the temperature of the room.

Allow this last to stand quietly, so that the clot may settle, leaving the clear serum above.

Experiment 1. Place a drop of the serum on a piece of red litmus paper. It turns blue, showing the blood serum to be alkaline.

Experiment 2. Saturate some of the serum with magnesium sulphate ($MgSO_4$). A precipitate of ser-globulin is produced. Filter this precipitate off, and keep the filtrate.

Experiment 3. Remove some of the substance collected on the filter, and place it in a test tube with water.

It dissolves, due to the salt retained by the precipi-

tate. Pure ser-globulin is insoluble in water, but dissolves in dilute salt solutions.

Experiment 4. Heat a portion of the above ser-globulin solution. It coagulates.

Experiment 5. Apply the Biuret test (see Experiment 8, Lesson 3) to another portion. A violet reaction will be obtained.

Experiment 6. Add some sodium sulphate (Na_2SO_4) to the cold filtrate obtained in Experiment 2. A precipitate of ser-albumin occurs. Collect the precipitate on a filter.

Experiment 7. Remove the precipitate from the filter, and place it in a test tube with water. It dissolves.

Heat a portion, and observe that it coagulates.

Apply Experiments 9, 10, 12, and 13, Lesson 3, to some of the solution. The relationship of ser-globulin to ser-albumin in human blood serum is 1:1.5, in oxen, 1:0.842, in dogs, 1:1.8.

Experiment 8. Wash some of the blood clot in water until nearly white and free from blood corpuscles.

Observe the thready or fibrous character of the fibrin.

Experiment 9. Apply Experiments 6 and 7, Lesson 3, to some of the fibrin, showing it to be a protein substance.

Experiment 10. Place a thread of fibrin in some hydrogen peroxide (H_2O_2). A rapid evolution of oxygen gas is the result. The oxygen may be tested for by its relighting a spark on the end of a match.

Experiment 11. Mix a drop of defibrinated blood of

a guinea pig on a slide with a drop of water, put on a cover-glass, and in a few minutes the corpuscles are rendered colorless, and then the oxyhæmoglobin crystallizes out as rhombic tetrahedra (four-sided pyramids). The form of the crystals varies with the blood from different animals.

Experiment 12. Treat a little of the hæmoglobin given with a drop or two of nitric acid (HNO_3) on a watch glass, and apply gentle heat to dryness. When cold, test the presence of iron in the residue by means of a drop of ammonium sulphocyanide (NH_4CNS), which gives a blood-red coloration in the presence of iron.

Experiment 13. Observe the estimation of hæmoglobin in a specimen of blood by means of a hæmoglobinometer.

Experiment 14. Make a dilute blood solution in a test tube, and place it in front of the slit of the spectroscope, allowing the light to pass through the solution.

An absorption spectra will be seen with two (2) absorption bands between the Fraunhofer lines D and E, namely, in the yellow and green parts of the spectrum.

This absorption is produced by the oxyhæmoglobin.

Experiment 15. To another portion of dilute blood add some Stoke's reducing solution (an ammoniacal solution of iron tartrate). Observe the change of color of the solution, and place it in front of the spectroscope as before.

An absorption spectra will be seen with only one (1) broad absorption band between the Fraunhofer lines D and E.

This absorption is produced by hæmoglobin or reduced oxyhæmoglobin.

Experiment 16. Place a drop of blood on a slide, and add a grain of common salt, and cover with a cover-glass. Now add some glacial acetic acid under the cover-glass, and warm gently until bubbles rise. When viewed with the microscope, dark brown, long rhombic crystals of hæmin are seen. If no crystals appear after the first heating, warm again, and, if necessary, add some more acetic acid. These crystals are sometimes called Teichmann's crystals, and are characteristic of blood-coloring matters.

Experiment 17. Add some tincture of guaiacum to some dilute blood solution in a test tube, and then a drop or two of hydrogen peroxide (H_2O_2). A bluish-green, and then a blue coloration is obtained. A frothing of the liquid is also observed, due to the evolution of oxygen from the hydrogen peroxide.

Experiment 18. Apply the same test to a blood stain on a piece of cloth. The same coloration is obtained as above.

BILE.

Experiment 19. *Pettenkofer's test* for bile acids. Treat a few drops of bile in a porcelain dish with a few drops concentrated sulphuric acid (H_2SO_4) and warm gently. Then add a few drops of a ten-per-cent. solution of cane sugar, continually stirring with a glass rod. A beautiful red liquid, which gradually changes to bluish violet, is obtained. This shows the presence of bile acids (glycocholic and tauracholic acids).

Experiment 20. *Gmelin's test* for bile pigments.

Place some yellow nitric acid (containing nitrous acid) in a test tube and allow some bile to flow down gently on its surface. A series of colored layers are obtained at the juncture of the two liquids in the following order from above downwards: green, blue, violet, red, and reddish-yellow. The green ring must never be absent, otherwise the reaction is not characteristic of bile pigments.

Experiment 21. *Huppert's test* for bile pigments.

Add some calcium chloride ($CaCl_2$) solution to some bile solution in a test tube and precipitate with ammonium hydrate (NH_4OH). Filter off the precipitate, wash with a little water, transfer while moist to a test tube, and treat with alcohol which has been acidified with sulphuric acid. Now boil for some time, when the solution becomes emerald-green or bluish-green in color.

Experiment 22. *Smith's test* for bile pigments.

Place some bile solution in a test tube and allow some tincture of iodine to flow on its surface. A green ring is seen at the juncture of the two liquids.

Experiment 23. Cholesterine.

Treat a crystal on a watch glass with a mixture of 5 parts sulphuric acid and 1 part water, when colored rings are obtained, first a bright carmine-red and then violet.

Lesson VI.

MILK.

Consists of an emulsion of very finely divided fat globules suspended in a solution of albuminous bodies, milk sugar, and salts in water.

Experiment 1. Test the reaction of the milk given by means of litmus paper. It may be amphoteric, that is, it may turn blue litmus paper red, and at the same time turn red paper blue, showing it to have both an acid and an alkaline reaction to litmus.

Experiment 2. Take the specific gravity of the milk by means of the hydrometer at a temperature of 15° C.

Pure milk should have a specific gravity of 1028 to 1034.

Experiment 3. Mix a little water with some milk (about $\frac{1}{4}$ its volume) and take the specific gravity at the same temperature as before. The specific gravity is diminished depending upon the amount of water added.

Experiment 4. Boil some of the milk in a test tube. No coagulation or change is observed, with the exception of the formation of a skin on the surface.

Experiment 5.—Slightly acidify a little of the milk with acetic acid and then warm. An immediate coagulation or curdling of the casein is produced.

Experiment 6. Warm some milk up to 40° C. and add some rennet powder or rennin. The casein coagulates immediately, forming a clot, and a clear liquid called the "whey" separates. Filter the liquid off and retain it for further experiments.

This process is made use of in the making of cheese.

Experiment 7. Heat a little of the clot on foil. It burns with an odor of burnt hair, showing it to be nitrogenized.

Experiment 8. Apply the Biuret test to a portion of the clot. A violet reaction is obtained showing it to be a protein body.

Experiment 9. Shake another portion of the clot in a test tube with some ether. Decant the ether into a watch glass, and let it evaporate. A residue of fats will be found in the watch glass. This shows that the casein in coagulating carries with it most of the fats of the milk.

Experiment 10. Add some copper sulphate ($CuSO_4$) solution to some milk in a test tube, and then one drop of caustic soda (NaOH) solution. A precipitate of the casein, carrying most of the fats with it, is produced. This is the basis of Ritthausen's method for the estimation of casein in milk.

Experiment 11. Heat some of the "whey" obtained in Experiment 6 with some Fehling's solution.

A copious reduction of copper sub-oxide shows the presence of milk sugar (lactose). The "whey" should have a sweetish taste.

Experiment 12. Witness the estimation of fat in a sample of milk by means of the Lactrocrit, and also by Soxhlet's extraction apparatus.

Experiment 13. Place some milk in a narrow test tube, and place it in the centrifugal machine and rotate for a few minutes. The cream, or fat, is made to rise to the surface, and should occupy from ten to fifteen per cent. of the column of the milk. The same result may be obtained by allowing the milk to stand in the test tube for twelve to twenty-four hours.

Experiment 14. Place a drop of the milk on a slide and cover with a cover-glass. View this under the microscope and observe the number and size of the fat globules.

Lesson VII.

URINE.

1. *Collection and measurement* of the twenty-four hours' urine. Start at a given time with an empty bladder, and then collect all the urine excreted in the following twenty-four hours.

Average normal amount for man about 1500 c. c. Women a little less. The quantity of drink greatly influences the quantity of urine voided.

2. *Color*, normally amber-yellow. (Refer to Vogel's color chart.)

3. *Transparency.* Clear, with the exception of a slight cloud of mucus, which suspends itself midway in the liquid on allowing it to stand.

4. *Consistency.* Not ropy.

5. *Odor.* Urinous, but sometimes certain foods and medicines give a characteristic odor.

6. *Reaction.* Normally slightly acid for the twenty-four hours. Portions voided at different times of the twenty-four hours and after certain food have different reactions. The reaction is determined by placing some of the urine on litmus paper. If it be acid, the blue paper will turn red. If alkaline, the red paper will turn blue.

The alkalinity may be due to the *fixed* alkalies, such

as sodium or potassium carbonates or phosphates, or to *volatile* alkali, such as ammonium carbonate.

If the alkalinity is due to the volatile alkali, on suspending the litmus paper, which has turned blue, to the air, the original red will come back again. If the alkalinity be due to the fixed alkalies, the blue color will be permanent.

Experiment. Test the reaction of the sample of urine given. If alkaline, determine if it is due to fixed or volatile alkalies, as above described.

Experiment. If the alkalinity is due to volatile alkali, it will have an odor of ammonia and will give white fumes of ammonium chloride (NH_4Cl) if a glass rod, moistened with hydrochloric acid, is held over the urine.

7. *Specific Gravity.* Normally about 1020 at 60° F., or 15.5° C. The specific gravity varies generally with the quantity of urine voided, but not always.

It is generally determined by means of a urinometer or specific gravity bottle. Always determine the specific gravity at 60° F. or 15.5° C., taking care not to have any air bubbles attach themselves to the instrument, and to have the vessel, holding the urine, large enough so that the instrument floats readily. The vessel should not be held by the hand, but should be allowed to stand in a vertical position. It should be filled to the top with urine.

Read off on the spindle of the instrument where the meniscus of the liquid strikes the graduation.

Experiment. Determine the specific gravity of the sample of urine given.

Experiment. Dilute a given volume of this urine

with an equal volume of water, and mix thoroughly. Take the specific gravity of the mixture. It will be found to be one half of the original urine. If the specific gravity of the mixture of one volume of urine and two volumes of water is taken, it will be found to be one third of the original urine.

In cases where too little urine is voided to take the specific gravity, the urine can be diluted with a known volume of water, and then the specific gravity taken, and from this calculate the specific gravity of the original urine.

8. *Total Solids.* The approximate amount of solids may be calculated from the specific gravity by multiplying the last two figures of the specific gravity when below 1018 by 2 or when above by 2.33. The result is parts of solid in 1000 parts of the urine.

Experiment. Make the calculation for the urine given, supposing in one case the total quantity of urine voided in the twenty-four hours was 1450 c. c. and in the other case 850 c. c.

Experiment. Heat some of the evaporated urine given on the foil. It blackens and burns with an odor of burnt hair, due to the nitrogenous organic matter. On continuing the heat all the organic matter burns away, leaving a white residue which consists of the inorganic salts.

NORMAL PROPORTIONS.

Total	solids....	60	grammes	in	the	24 hours.
Organic	"	35	"	"	"	" "
Inorganic	"	25	"	"	"	" "

Organic Solids.		*Inorganic Solids.*	
Urea	30 grms.	Sodium chloride	15 grms.
Uric acid	0.7 "	Sulphuric acid	2.5 "
Creatinin	1.0 "	Phosphoric acid	2.5 "
Hippuric acid	0.7 "	Potash	3.3 "
Remaining organic bodies	2.6 "	Ammonia	0.7 "
		Magnesia	0.5 "
		Lime	0.3 "
		Remaining inorganic bodies	0.2 "

UREA, CH_4N_2O.

Colorless, crystalline needles or prisms.

Very soluble in water or alcohol. It is soluble in its own weight of water and five times its weight of alcohol.

Experiment. Try the solubility of the urea given in water. Retain this solution for further use.

Experiment. Try the solubility of the urea in alcohol. Place a drop of this alcoholic solution on a clean microscope slide and observe the crystallization.

Urea is a basic compound forming salts with acids, such as urea nitrate CH_4N_2O,HNO_3 or urea oxalate, CH_4N_2O, $C_2H_2O_4$, which are crystalline.

Experiment. Place a little of the watery solution of urea obtained above in a watch glass and add a few drops of nitric acid thereto. Hexagonal crystalline plates of urea nitrate are formed in the liquid.

This compound may be employed in the detection of small amounts of urea in liquids.

Experiment. Treat some of the evaporated urine

given with a few drops nitric acid and observe the formation of the crystals of urea nitrate.

Urea readily changes into ammonium carbonate by the action of chemical processes or micro-organisms. (This latter is called alkaline fermentation of the urine.)

$$CH_4N_2O + 2H_2O = (NH_4)_2CO_3.$$

This change may take place in the body and the urine voided under such circumstances will be alkaline due to volatile alkali, will probably have an odor of ammonia and give white fumes of ammonium chloride with a drop of hydrochloric acid, if held over it on a glass rod.

Experiment. Heat a little urea in a dry test tube. It melts, decomposes, and gives off ammonia (NH_3), and leaves finally a white mass, which if dissolved in water and treated with a *drop* of copper sulphate ($CuSO_4$) and an excess of caustic soda, gives a beautiful reddish-violet liquid. (Biuret reaction.)

Urea is decomposed by an alkaline solution of sodium hypochlorite, or, better, hypobromite into nitrogen, carbon dioxide, and water, according to the following equation:

$$CH_4N_2O + 3NaBrO = N_2 + CO_2 + 2H_2O + 3NaBr.$$

Experiment. Place a little urea solution in a test tube and add some sodium hypobromite. A violent decomposition takes place with the rapid evolution of gas. Let the gas bubbles subside and apply a lighted match to the gas in the test tube. The flame is extinguished, due to the nitrogen, which is a non-supporter of combustion. The carbon dioxide (CO_2) evolved is absorbed by the alkaline solution of sodium hypobromite.

Experiment. Quantitative Estimation. Fill the *ureometer* with sodium hypobromite (prepared as described below) by holding it in a vertical position and pouring the solution into the bulb, and when it is rather more than one half full, incline the instrument horizontally until the entire tube is filled and a little left in the bulb, say about one third, when in the vertical position. Now fill the nipple pipette up to the I c. c. mark with urine and *slowly* inject this amount of urine up under the tube and *not* in the bulb of the instrument. Allow frothing to cease and let it stand a moment or two, so that the carbon dioxide may be absorbed. Read off on the graduation the weight of urea corresponding to the volume of nitrogen collected. The instruments are graduated to read fractions of a gramme of urea per cubic centimeter of urine.

The solution of sodium hybromite is prepared as follows:

Dissolve 100 grammes caustic soda in 250 c. c. water, and adding 25 c. c. bromine to this solution when cold. For use in the ureometer this solution should be diluted with an equal volume of water.

As this solution does not keep for any length of time, a solution has been suggested by Dr. Rice as follows:

1. Dissolve 125 grammes bromine, 125 grammes sodium bromide in 1000 c. c. water.

2. Prepare a solution of caustic soda of a specific gravity of 1.250.

For use in Doremus's ureometer mix 5 c. c. of each of the two above solutions and dilute with 15 c. c. water.

URIC ACID $C_5H_4N_4O_3$ or $H_2C_5H_2N_4O_3$.

Is a bibasic acid, thus forming two series of salts. Example:

Acid sodium urate, $NaHC_5H_2N_4O_3$.
Neutral " " $Na_2C_5H_2N_4O_3$.

Soluble with difficulty in cold water (1 part in 15,000 parts) and more soluble in boiling water (1 part in 1900 parts). The acid salts are less soluble than the neutral salts. The alkali salts are more soluble than the earthy, such as calcium or magnesium. Ammonium urate is very insoluble. The uric acid exists in the urine as urates and never free. Sodium phosphate is considered as the solvent for the uric acid in the urine.

Uric acid may occur in nearly every crystalline form, but generally as small rhombical prisms or plates, dumb-bell shaped crystals, whetstone shaped, etc. When deposited from the urine they are always more or less colored.

Experiment. Place a little uric acid in a test tube and add some water. Note insolubility. Heat to boiling and notice the apparent insolubility.

Experiment. Add a few drops of sodium carbonate to the above solution. The uric acid dissolves readily with the formation of sodium urate.

Experiment. Add a few drops of hydrochloric acid to a portion of the above solution of sodium urate. A white precipitate of uric acid is produced. The HCl added combines with the sodium, setting the insoluble uric acid free.

Experiment. Saturate some of the solution of uric acid in sodium carbonate with ammonium chloride. A

white gelatinous precipitate of insoluble ammonium urate is formed. (This is the basis of Hopkin's method of estimating uric acid in urine.)

Experiment. Murexid test. Place a few grains of the uric acid on a watch glass, moisten with a drop of nitric acid, and gently heat to dryness over flame. A yellowish-red residue is obtained. When cold add a drop of ammonium hydrate. A purple coloration is the result, which becomes darker on the addition of caustic soda. The purple color is ammonium purpurate.

Experiment. Schiff's test. Dissolve a little of the uric acid in water, to which some sodium carbonate (Na_2CO_3) has been added. Add a drop of silver nitrate ($AgNO_3$) to a portion of the solution. A black reduction of silver oxide is obtained. The test may also be performed by applying a drop of the solution of uric acid to a piece of paper previously moistened with silver nitrate ($AgNO_3$). A black stain appears on the paper.

Experiment. Place a little Fehling's solution in a test tube, and add a little of the uric acid solution. A white precipitate of copper urate is produced, which if heated is reduced to red copper sub-oxide. This should be considered in testing for sugar (dextrose) in the urine. Alkaline solutions of bismuth are not reduced by uric acid.

Experiment. Place some uric acid on foil and apply heat. It blackens, gives off an odor of burnt hair, and is entirely consumed, showing it to be entirely of an organic nitrogenous nature.

Experiment. Heat some ammonium urate ($(NH_4)_2$

$C_5H_2N_4O_3$) on foil. It blackens, gives off an odor of burnt hair, and entirely disappears.

Experiment. Heat some potassium urate ($K_2C_5H_2N_4O_3$) on the foil. It blackens, gives off an odor of burnt hair, and leaves a residue of potassium carbonate (K_2CO_3), which, if placed on moistened red litmus paper, turns it blue because of its alkalinity.

Quantitative Estimation. Acidify 200 c.c. of the urine previously filtered and free from albumin, with 10 c.c. hydrochloric acid in a beaker, stir and allow this mixture to stand in a cool place for twenty-four to forty-eight hours. Filter off the precipitated uric acid through a previously weighed filter, taking care that all the particles of uric acid are removed from the sides of the glass vessel and transferred to the filter. Wash the precipitate with water until it is free from chlorine, as shown by adding silver nitrate to a portion of the filtrate. Now dry and weigh.

As some uric acid is dissolved by the wash water, the filtrate and wash water should be measured, and for each 10 c.c. of filtrate and wash water 0.00048 grms. uric acid must be added to the amount found by weighing.

Hopkins' Method. Treat 100 c.c. of the urine in a beaker with 30 grms. powdered ammonium chloride (NH_4Cl), and allow to stand in a cool place for twenty-four hours. Filter the precipitated ammonium urate through a small filter, and wash with a cold saturated solution of ammonium chloride. Puncture the filter and wash the precipitate into a beaker with a small amount of boiling water, and treat with 5 c.c. dilute hydrochloric acid and heat on the water bath. When

cold filter the uric acid through a weighed filter and proceed as above described.

HIPPURIC ACID, $C_9H_9NO_3$.

Crystallizes in semi-transparent, milk-white, long, four-sided rhombic prisms or columns, or in needles on rapid crystallization.

Hippuric acid dissolves in 600 parts cold water and more easily in hot water. It is soluble in alcohol. It is a monobasic acid forming hippurates with bases, which are soluble in water and alcohol.

Experiment. Boil a watery solution of hippuric acid with a drop or two of hydrochloric acid. It is split into benzoic acid (C_6H_5COOH) and glycocoll ($C_2H_5NO_2$). This change may also be brought about by certain micro-organisms.

Experiment. Add some ferric chloride (Fe_2Cl_6) to some of the above solution. A bluish-red coloration shows the presence of benzoic acid.

Experiment. Heat some of the hippuric acid (not solution) with nitric acid (HNO_3) in a test tube. An odor of oil of bitter almonds (nitro-benzole) is developed.

Other organic bodies occurring in the urine:

Creatinin	$C_4H_7N_3O$
Oxalic acid	$C_2H_2O_4$
Allantoin	$C_4H_6N_4O_3$
Xanthin	$C_5H_4N_4O_2$
Hypoxanthin	$C_5H_4N_4O$
Guanin	$C_5H_5N_5O$
Adenin	$C_5H_5N_5$

Lesson VIII.

URINE.

Occur chiefly as sodium chloride (NaCl) with small amounts of potassium chloride. They are estimated as sodium chloride. Sodium chloride is very soluble in cold and hot water, hence it never occurs in urinary sediments or calculi.

Experiment. Add some silver nitrate to some dilute hydrochloric acid in a test tube. A white precipitate of silver chloride (AgCl) is produced. Divide into two portions. Add ammonium hydrate (NH_4OH) to one portion and notice that the precipitate dissolves. Add nitric acid (HNO_3) to the other portion. It does not dissolve.

Experiment. Place some sodium phosphate (Na_2HPO_4) in a test tube and add some silver nitrate ($AgNO_3$). A yellow precipitate of silver phosphate is formed. Add nitric acid (HNO_3) to this and observe that the precipitate is dissolved.

Experiment. To detect chlorides in a sample of urine, first add a few drops nitric acid and then silver nitrate, so as to prevent the phosphates from being precipitated by the silver nitrate, and in the presence of chlorides a white precipitate is obtained.

Quantitative Estimation. Place 20 c.c. of the urine

in a clean porcelain dish and add thereto, drop by drop, a standard solution of silver nitrate (see below) from a burette, constantly stirring with a glass rod. From time to time place a drop of the contents of the dish on a piece of paper previously moistened with a solution of potassium chromate. When a faint red stain is obtained on this paper stop adding the silver nitrate solution. This indicates that enough silver nitrate solution has been added to combine with all the chlorine in the 20 c. c. of urine in the dish. Read off the number of cubic centimetres of silver nitrate solution used. Each c. c. of the standard solution of silver nitrate is equal to 0.01 grammes sodium chloride (NaCl). From the weight of NaCl in the 20 c. c. of urine calculate the total amount in the twenty-four hours.

The standard solution of silver nitrate is prepared by dissolving 29.075 grammes fused silver nitrate in 1000 c. c. of distilled water. Each c. c. = 0.01 grms. NaCl.

SULPHURIC ACID, SO_3.

The sulphuric acid of the urine occurs either in combination with bases as potassium (K_2SO_4) and sodium sulphate (Na_2SO_4) or as ethereal sulphates, such as potassium phenol sulphate ($C_6H_5.O.SO_2.OK$), or potassium indoxyl sulphate ($C_8H_6N.O.SO_2.OK$).

Chiefly formed from the sulphur of the protein bodies by oxidation and a small portion from the sulphates of the food.

Experiment. Place some magnesium sulphate ($MgSO_4$) in a test tube and add some barium chloride

($BaCl_2$) thereto. A white precipitate of barium sulphate ($BaSO_4$) is produced.

Try the solubility of this precipitate of barium sulphate in nitric acid and in caustic soda. It will be found insoluble in both.

Experiment. Acidify some urine with acetic acid and add some barium chloride. A white precipitate of barium sulphate is produced. Filter this off and retain the filtrate.

This precipitate represents the sulphuric acid existing as potassium and sodium sulphates.

Experiment. Add some hydrochloric acid to the above filtrate and boil for a few minutes. This decomposes the ethereal sulphates setting more sulphuric acid free, and this is then precipitated as white barium sulphate by the barium chloride in the filtrate.

Quantitative Estimation. 100 c. c. of the filtered urine are acidified with 5 c. c. concentrated hydrochloric acid and boiled for fifteen minutes. While boiling add 2 c. c. of a strong solution of barium chloride and warm for a little while until the barium sulphate has completely settled. Filter through a small filter and wash with hot water and then with alcohol (to remove any resinous bodies). Dry the filter, incinerate and weigh according to prescribed methods. From the weight of barium sulphate calculate the amount of sulphuric acid in the urine used.

PHOSPHORIC ACID, P_2O_5.

It occurs in urines combined with sodium, potassium, ammonium, calcium, and magnesium, and as phosphoric acid is tribasic, three series of salts occur, namely acid,

neutral, and basic phosphates. The phosphates of potassium, sodium, and ammonium are called *alkali* phosphates, and are very soluble in the urine; while the phosphates of calcium and magnesium are called *earthy* phosphates and are difficultly soluble in acid urine, but insoluble in alkaline urines.

The chief quantity of phosphoric acid is derived from the phosphates of the food, but a small amount is derived from the oxidation of organic bodies containing phosphorous such as nuclein, lecithin, protagon.

Experiment. Place some sodium phosphate (Na_2HPO_4) in a test tube with water and add some magnesium sulphate ($MgSO_4$) solution, then some ammonium chloride (NH_4Cl) and lastly some ammonium hydrate (NH_4OH). A copious white precipitate of magnesium-ammonium phosphate ($MgNH_4PO_4,6H_2O$, triple phosphate) is formed. This is a good test for phosphoric acid or phosphates.

Experiment. Add some caustic soda (NaOH) to some urine in a test tube and warm gently. A flocculent precipitate of earthy phosphates is produced.

Filter this precipitate off and make the filtrate acid with acetic acid, add magnesium sulphate, ammonium chloride, and then ammonium hydrate. A white precipitate of magnesium-ammonium phosphate is formed, derived from the alkali phosphates in the urine.

Experiment. Add some ammonium hydrate to some urine in a test tube. A white precipitate of calcium phosphate ($Ca_3(PO_4)_2$) and crystalline magnesium-ammonium phosphate (triple phosphate) is formed. This forms whenever urine becomes ammoniacal, by decomposition of urea, on standing.

Experiment. Add some uranium acetate solution to some sodium phosphate in a test tube. A white precipitate of uranium phosphate is obtained. This is one of the best tests for phosphoric acid or phosphates.

Quantitative Estimation. Place 50 c. c. of the filtered urine in a clean porcelain dish and add a few drops acetic acid or better a solution of sodium acetate. Warm over water the bath and add a standard solution of uranium acetate from a burette drop by drop, stirring all the while, until a drop taken out and placed on a piece of paper moistened with potassium ferrocyanide ($K_4Fe(CN)_6$) gives a faint chocolate brown stain. Place it on the water bath again and warm and apply a drop to the potassium ferrocyanide paper. If the stain is permanent stop adding the uranium acetate solution, otherwise go on until a permanent stain is obtained. Each c. c. of the standard solution of uranium acetate corresponds to 0.005 grammes phosphoric acid (P_2O_5). From the above result calculate the total amount of phosphoric acid in the twenty-four hours urine.

Experiment. To determine the P_2O_5 in combination as alkali or earthy phosphates separately, first precipitate the earthy phosphates from 100 c. c. of the urine by the addition of a very small amount of ammonium hydrate. Filter this precipitate off and collect 50 c. c. of the filtrate. Acidify this with acetic acid and determine the quantity of P_2O_5 therein by means of the standard solution of uranium acetate as above directed. This gives the amount of P_2O_5 combined with sodium and potassium or alkali phosphates. On subtracting this result from the total amount of P_2O_5 in the urine

we get the amount of P_2O_5 existing in combination with calcium and magnesium, or earthy phosphates.

ABNORMAL CONSTITUENTS.—PROTEIN SUBSTANCES.

Abnormal urine may contain ser-albumin, ser-globulin, peptones, mucin, fibrin, etc., but the most common substance is ser-albumin.

SER-ALBUMIN.

In testing the presence of albumin in a sample of urine it should be perfectly clear, and if not, it must first be clarified by filtration through one or more folds of filter paper. If it cannot be clarified by simple filtration (due to the presence of micro-organisms), add some magnesium sulphate ($MgSO_4$), ammonium chloride (NH_4Cl), and then some ammonium hydrate (NH_4OH), forming a precipitate of triple phosphate, which retains the bacteria, and which may be removed by filtration. The filtrate must be made acid with acetic acid before applying the tests for albumin.

Before applying the tests to a sample of urine first test the reaction of the urine, and if alkaline make it neutral or slightly acid with acetic acid.

Experiment. Fill a test tube two thirds full with some of the clear urine and apply heat to the upper layer of the liquid, holding the test tube at the bottom. A precipitation or coagulation may occur, but it may be due to earthy phosphates or albumin, or both, because they are precipitated by heat alone.

Now add three (3) drops of nitric acid to the liquid. If the precipitate dissolves completely on the addition of the acid it is due to earthy phosphates, but if it does

not, and the urine remains opalescent and cloudy, it contains albumin. Boil again and observe if the precipitate increases.

Experiment. Heller's test. Place about one half an inch of nitric acid in a test tube and allow the urine to flow gently on the surface of the acid without mixing with it. This may be done by allowing the urine to flow from a filter, holding the point of the funnel on the side of the test tube, inclining it slightly. The urine may also be floated on the surface of the acid by means of a nipple pipette, holding the test tube in an inclining position.

If albumin is present a sharply-defined opalescent ring or zone will be observed at the *point of contact* between the two liquids. In the presence of traces of albumin some time is required for the development of the zone. With a urine containing an excess of uric acid a zone is obtained, but it is higher up in the liquid and not sharply defined as the albumin zone.

The ring due to coloring matters should not be confounded for the albumin zone.

Experiment. Fill a test tube one half full of clear urine and add some clear potassium ferrocyanide ($K_4Fe(CN)_6$) solution and mix thoroughly. Now add ten drops acetic acid, and if albumin is present a white cloudiness or precipitate is obtained. The obtainment of a cloudiness with this test as above directed shows the presence of albumin and nothing but albumin.

It is advisable in all these tests, in the presence of faint traces of albumin, to have a second test tube containing the same amount of clear urine, without any reagents, and hold the two test tubes side by side and

look through the liquid at a dark background. By this means the faintest cloudiness may be detected in the test tube to which the reagents have been added by difference in appearance.

Experiment. Tanret's test. Acidify some of the urine in a test tube with a few drops of acetic acid, and add a few drops of a solution of double iodide of mercury and potassium. A white cloudiness or precipitate is obtained in the presence of albumin. This test may also be applied by the contact method; the reagent is placed first in the test tube, and then the urine allowed to float on top. A white ring or zone at the point of contact of the two liquids is the result in the presence of albumin.

The reagent is prepared by adding potassium iodide to some mercuric chloride solution in a test tube and just re-dissolving the red precipitate of mercuric iodide formed by a slight excess of potassium iodide solution.

This test is very delicate, but peptones, mucin, vegetable alkaloids also respond to it. The precipitate obtained with peptones and alkaloids is dissolved by the application of a gentle heat and reappears on cooling again.

Experiment. Robert's Nitric-Magnesium test. Float the urine on the surface of a solution of magnesium sulphate containing nitric acid. In the presence of albumin a sharply-defined zone or ring is seen between the two liquids.

The solution is made by adding one ounce of strong nitric acid to five ounces of a saturated solution of magnesium sulpate. This modification is said to be more delicate than Heller's test.

Experiment. Acidify some of the clear urine with acetic acid and add a few drops of a picric acid solution. A turbidity or cloudiness is seen as the picric acid diffuses through the liquid.

Peptones, alkaloids, such as quinine, morphine, etc., are precipitated by this reagent, but the precipitate disappears on the application of heat and reappears on cooling again.

Experiment. Make some of the urine acid with acetic acid, and add a few drops of a solution of sodium tungstate. In the presence of albumin a cloudiness or precipitate is observed. In addition to albumin this reagent precipitates peptones and mucin but not the alkaloids. The precipitate obtained with peptones is dissolved by gentle heat.

Quantitative Estimation. Heat 50 c.c. water containing 10 c.c. acetic acid to boiling, and add gradually, while boiling, 100 c.c. of the clear urine, and boil for a few minutes to insure complete precipitation. Filter through a weighed filter, wash with hot water until free from acid; dry, and weigh.

Esbach's Method. Fill the albuminometer tube to the letter U with the clear urine, and then to the letter R with the test solution. (See below.) Close the end of the tube with a rubber stopper, invert several times, and allow the mixture to stand twenty-four hours. When the precipitate has completely settled, read off on the graduation on the tube the number of grammes of ser-albumin per litre of urine. The percentage is derived from this by moving the decimal point one figure to the left; thus 4 grammes per litre would be 0.4 per cent. of albumin. If the urine contains con-

siderable albumin, the urine must first be diluted with one or two volumes of water before testing, and the result multiplied by two or three, as the volume is doubled or trebled.

The test solution is prepared by dissolving 10 grammes picric acid and 20 grammes citric acid in 1000 c.c. distilled water. In order to save time the tube may be placed in a centrifugal machine, and the albumin precipitated in three or four minutes.

MUCIN.

Mix some saliva with some normal urine and apply the following tests:

Experiment. Dilute some of this urine in a test tube with water, and acidify with an excess of acetic acid. A precipitate of mucin is formed. This precipitate dissolves on the addition of caustic soda, and is again precipitated on the addition of an excess of acetic acid.

Experiment. To another portion add some acetic acid, and then some potassium ferrocyanide. No cloudiness or precipitate.

Experiment. Repeat Heller's test, with some of the urine containing mucin. A zone or ring is obtained, but it is not so sharply defined, and is not at the point of contact of the two liquids.

Experiment. Treat the urine with an equal volume of a saturated solution of common salt, and then add a solution of tannic acid, prepared by dissolving 5 grammes tannic acid in a mixture of 10 c.c. 25 per cent. acetic acid and 240 c.c. 40–50 per cent. alcohol.

In the presence of the smallest amounts of mucin a precipitate is formed.

PEPTONES.

Add a little peptone to some normal urine and use this for the following test :

Experiment. Saturate some of the urine with ammonium sulphate after having slightly acidified it with acetic acid. This precipitates the albumin, globulin, and albumoses, leaving the peptones in solution. Filter and test the filtrate for peptones by the Biuret test, Tanret's test, and with picric acid.

Lesson IX.

CARBOHYDRATES.

Dextrose. Urine containing sugar as a rule has a high specific gravity, a light color, and is large in amount, sometimes three or four times the normal amount.

Before testing the urine for sugar see that it does not contain any protein substances, otherwise they must first be removed by coagulation with heat and acetic acic and filtration. Albumin interferes with the tests for sugar.

Experiment. Moore's test. Add some caustic soda to some urine in a test tube, and apply heat to the upper part of the liquid. In the presence of sugar the urine becomes yellowish-brown in color, as seen by observing the difference in the color between the heated and the unheated portions. The depth of color depends upon the quantity of sugar present.

The color disappears on the addition of a few drops of nitric acid, and develops an odor of burnt sugar (caramel).

This test is best applied to decolorized urines.

Bile pigments produce a brown color with caustic soda without the application of heat.

Experiment. Trommer's test. Treat some of the urine with about one fourth its volume of caustic soda, and

then, while shaking, add a 10 per cent. copper sulphate solution until the precipitate formed just redissolves, and a deep blue solution is the result. Apply a gentle heat to the upper part of the liquid and a reduction of yellow hydrated cuprous oxide, and then red cuprous oxide is obtained. It should not be boiled too long, neither should the precipitate of earthy phosphates be mistaken for a reduction of cuprous oxide, which settles quickly to the bottom of the test tube.

Experiment. Fehling's test. The solution as suggested by Fehling does not keep, hence it is advisable to make two solutions and keep them separated. 1. Dissolve 34.65 grammes pure copper sulphate in 1000 c. c. water. 2. Dissolve 173 grammes Rochelle salt in 350 c. c. water, adding 600 c. c. of a caustic soda solution of a specific gravity of 1.12 and dilute to 1000 c. c. with water. For use mix equal parts of the above solutions and dilute with an equal volume of water.

Glycerine may be used instead of the Rochelle salt.

In applying this solution to the urine first fill a test tube one half full of the solution and boil for a few seconds. No change or reduction should be observed in the liquid. Now add the urine drop by drop and if sugar is present a reduction of yellow hydrated cuprous oxide and then a red cuprous oxide is seen. The test should not be boiled after the addition of the urine but only warmed.

If a reduction occurs in the test solution alone on boiling it must be discarded.

Urines containing large amounts of uric acid, creatinin, hippuric acid, hypoxanthin, allantoin, alkaloids, glycuronic acid, etc., may give a marked reduction with

these preceding tests and may lead to error. After the administration of certain drugs such as benzoic acid, turpentine, chloral, glycerin, salicylic acid, etc., a reducing substance called glycuronic acid is eliminated in the urine, which may mislead the experimenter. Urines containing an excess of ammonia, should be boiled, to drive off the ammonia, before applying the above tests as the ammonia has a tendency to dissolve any cuprous oxide precipitated.

Experiment. Boettger's test. Make some of the urine alkaline with sodium carbonate and add a little solid bismuth sub-nitrate. Heat to boiling and in the presence of sugar the white bismuth sub-nitrate will turn gray, brown or black, depending upon the amount of sugar present. The sugar reduces the bismuth sub-nitrate into bismuth oxide and metalic bismuth (?).

Experiment. Nylander's modification of Boettger's test. Dissolve 4 grammes Rochelle salt in a solution of 10.33 grammes caustic soda in 100 c. c. water. Add to this 2 grammes bismuth sub-nitrate and digest on the water bath until as much of the bismuth salt has dissolved as possible.

Heat 10 vols. of the urine with 1 vol. of the above solution for a few minutes when a black precipitate or a dark coloration is the result.

Before applying either of the two preceding tests any albumin present must first be removed, otherwise the sulphur of the albumin will turn the bismuth salt black.

Bismuth sub-nitrate is not reduced by the substances mentioned in connection with Trommer's or Fehling's test.

Experiment. v. Jaksch's test. Place 25 c. c. of the urine in a small flask and add 1 gramme (15 grains) phenyl-hydrazine hydrochloride and 0.75 grammes (10 grains) sodium acetate. Warm on the water bath for at least one hour. Allow to cool and the presence of sugar is shown by the presence of a yellowish deposit, which may appear amorphous to the naked eye but which, when examined under the microscope, is seen to contain fine, bright yellow needle-like crystals, either single or in stars. These crystals of phenyl-glucosazone melt at 204° C., and are highly characteristic and cannot be mistaken for any other substance.

This test does not give any reaction with uric acid, creatinin hippuric acid, or any of the other reducing substances mentioned above. It is one of the most delicate and most reliable of all known tests for sugar in the urine.

Experiment. Make some of the urine alkaline with caustic soda, add some picric acid solution and heat the upper part of the liquid. In the presence of sugar a dark mahogany-red color will be produced. This test has its interferences, namely creatinin gives a red coloration with this test.

Experiment. Fermentation test. Shake a little piece of yeast with some of the urine and fill this into an Einhorn Saccharometer and place this in a warm locality (40° C.) for 12 hours. The dextrose will ferment with the evolution of carbon dioxide, which will collect in the tube of the instrument.

This test may also be performed by simply shaking some yeast with four ounces of the urine in a bottle which is tightly corked and allow it to stand in a warm

place for 12 hours. If sugar is present, on removing the cork partly, it will be forcibly ejected and the carbon dioxide will escape as a froth. If much sugar is present the bottle may burst.

The fermentation test is the most reliable test for dextrose in the urine and is readily performed. If a urine responds to the reduction tests and does not ferment with yeast, it is safe to conclude that no dextrose is present, but contains other substances which have a reducing action. (Lactose gives the reduction tests but does not ferment.)

Quantitative Estimation. Experiment. Robert's differential desity method. Take the specific gravity of the urine at a known temperature, and then add a little yeast and allow it to ferment in a warm place for 24 hours. Take the specific gravity again at the same temperature as before. Each degree of specific gravity lost represents 1 grain of dextrose per fluid ounce of the urine, or the percentage may be ascertained by multiplying each degree of specific gravity lost by 0.23. In taking the specific gravity, be careful not to have any gas-bubbles attach themselves to the urinometer; otherwise an incorrect reading will be taken.

Einhorn's Saccharometer. Fill one of the instruments with some of the urine to be tested containing a little yeast, and the second instrument with normal urine containing the same amount of yeast. Place the two side by side in a warm place for twelve hours. Read off on the instrument the percentage of sugar from the volume of gas collected. The object of the second instrument is to see if the yeast itself gives off any gas. These results are only approximate, as no account is taken of the gas dissolved by the urine itself.

Fehling's Titration Method. Place 20 c. c. of the standard Fehling's solution (prepared as above described) in a flask, and dilute with two volumes water. Heat to boiling and add small amounts of the urine from a burette until the blue color of the solution has entirely disappeared. After each addition of urine from the burette it is advisable to boil the contents of the flask and allow it to stand a few seconds so that the reduced cuprous oxide may settle, and so the observer may see if the blue color has entirely disappeared. When the blue color has disappeared, read off the number of cubic centimeters of urine used from the burette. Since it requires 0.05 grammes dextrose to remove the blue color (namely all the copper) from 20 c. c. of Fehling's solution, therefore the quantity of urine used must have contained 0.05 grammes dextrose. From this calculate the percentage of dextrose in the urine.

This operation should be repeated to get exact results. If the original urine contains a large amount of dextrose, then dilute it first with a known volume of water.

Polariscope. Dextrose has a right-handed or dextrorotatory action on polarized light. Its specific rotatory power is $(a)D = +52.6$. In using this instrument for the determination of the amount of sugar in a urine, first decolorize the urine by shaking it with freshly burnt animal charcoal, filter and place the perfectly decolorized and clear urine in the glass tube provided for the purpose. Place this later between the polarizer and the analyser, and rotate the analyser until both halves of the field of view are equally illuminated. Read off

on the scale by means of the vernir the percentage of dextrose in the urine.

Urines may also contain:

Lactose, in the urine of women when nursing.

Inosite.

Levulose, occasionally in diabetic urines.

Maltose.

BILE.

Urine containing bile pigments has a color varying from bright yellow to a greenish-brown, and when shaken, readily gives a foam having a yellow color.

It is generally ropy, with a high specific gravity.

Experiment. Apply Gmelin's test to the urine as described in Lesson 5, Experiment 20. The test should be allowed to stand some little time so as to allow the characteristic rings of color to develop.

Experiment. Apply Huppert's test, as described in Lesson 5, Experiment 21, to the urine.

Experiment. Repeat Smith's test as described in Lesson 5, Experiment 22.

The bile acids can only be tested for in a urine with great difficulty.

BLOOD.

Blood occurs in urines in cases of *hæmaturia* where we have the presence of blood corpuscles, and in cases of *hæmoglobinuria* where we find the blood pigments with very few corpuscles. The presence of blood in a sample of urine may be seen by its characteristic color and its smoky and dichroic appearance. The presence of blood corpuscles is determined by microscopic examination.

Experiment. Test the presence of albumin in the urine containing blood.

Experiment. Make some of the urine alkaline with caustic soda and boil so as to precipitate the earthy phosphates. They will be colored bright red, due to their retaining blood pigments. Collect this precipitate on a filter and use it for the obtainment of Hæmin or Teichmann's crystals, as described in Lesson 5, Experiment 16. In this case there is no need of adding any common salt.

Experiment. Repeat Experiment 17, Lesson 5, with the urine, but instead of using hydrogen peroxide, use ether which has been ozonized (by shaking it with hydrogen peroxide). As the ether floats on the mixture of urine and tincture of guaiacum it forms a blue ring in the presence of blood.

Experiment. Slightly acidify the urine with acetic acid, filter and place it in front of the slit of the spectroscope. Two absorption bands will be seen, as described in Lesson 5, Experiment 14.

PUS.

Urine containing pus corpuscles has a cloudy appearance and is clarified by filtration with difficulty.

Microscopical examination reveals the presence of pus corpuscles.

Experiment. Test for albumin in the urine containing pus. It will be present in large amounts.

Experiment. Allow the corpuscles to settle or facilitate by means of a centrifugal machine and treat them, after decanting the urine from them, with a small piece of caustic soda and stir. After a moment or two the

corpuscles are converted into a ropy and gelatinous mass.

Experiment. Acidify the urine with acetic acid, filter and treat a portion of the contents of the filter with a few drops of tincture of guaiacum (which has been kept in the dark), when in the presence of pus the filter paper is colored a deep blue.

Experiment. Place some of the contents of the filter obtained above on a slide and treat it with a drop of a solution of iodine in water containing some potassium iodide. In the presence of pus a dark, mahogany-brown color, due to the glycogen contained therein, is the result. Epithelium cells do not give this color.

UROBILIN.

This is the normal coloring matter of the urine. It is markedly increased in fevers.

Experiment. Make some of the urine alkaline with ammonium hydrate, and filter off the precipitate of earthy phosphates. To the filtrate add a few drops of a solution of zinc chloride, which produces a beautiful greenish fluorescence by reflected light. The above solution exhibits, with the spectroscope, a dark absorption band between the Fraunhofer's lines b and F.

INDICAN.

This substance is derived from the indol, produced in the intestine by putrefactive processes, which is oxidized into indoxyl in the blood; this, entering in combination with sulphuric acid, is eliminated in the urine as sodium or potassium indoxyl sulphate or indican, ($KO.C_8H_6N.O.SO_2$).

Experiment. Treat a small amount of the urine in a test tube with an equal volume of hydrochloric acid and two or three drops sodium hypochlorite solution, and then a small amount of chloroform. Shake and put aside for a little while. The indigo set free from the indican is taken up by the chloroform, coloring it blue to a greater or less extent, depending upon the amount of indican present in the urine.

Experiment. Boil equal volumes of the urine and hydrochloric acid, containing a few drops nitric acid; allow to cool and shake with a little chloroform. The chloroform is colored violet, due to the setting free of indigo blue and indigo red from the indican.

Lesson X.

SEDIMENTS (Chemical or unorganized).

From acid urines.	From neutral or slightly alkaline (fixed) urines.
Uric acid.	Sodium urate.
Sodium urate.	Calcium oxalate.
Calcium oxalate.	Calcium carbonate.
Acid calcium carbonate.	Di-calcium phosphate.
	Tri-calcium phosphate.
	Ammonium-magnesium phosphate.

From ammoniacal urines.

Ammonium urate.
Ammonium-magnesium phosphate.
Tri-calcium phosphate.
Calcium carbonate.

In testing the sediments the urine should be allowed to stand in a conical vessel in a cool place until all the sediment has settled to the bottom. It may be collected by decantation, or better, by means of a pipette. Hold the first finger over the end of the pipette and introduce it to the bottom of the vessel into the sediment. Now remove the finger slowly and the sediment will rise in the tube. Close the end of the tube tightly again with the finger and remove it from the vessel. Allow the contents of the pipette to flow on a few

pieces of filter paper, which absorbs the urine, leaving the sediment on the paper, which may be removed by the aid of a knife or spatula, and tested as described below.

The sediment may also be obtained readily by means of a centrifugal machine.

URIC ACID, $C_5H_4N_4O_3$.

Generally colored red.

Experiment. Heat some on a piece of platinum foil. It blackens and burns up entirely, leaving no residue.

Experiment. Place a little in water in a test tube. It does not dissolve. When a drop or two of sodium carbonate is added it dissolves readily.

Experiment. Apply the Murexid test to a portion of the sediment. A purple coloration shows the presence of uric acid. (See uric acid.)

Experiment. Repeat Schiff's test with the solution obtained above. A black reduction denotes the presence of uric acid. (See uric acid.)

SODIUM URATE, $Na_2C_5H_2N_4O_3$.

Experiment. Heat a little on the foil. It blackens and burns with a yellow color to the flame, if looked at through a piece of blue glass. It does not burn up entirely, but leaves a residue, which if placed on a piece of moistened red litmus paper turns it blue, due to the sodium carbonate in the residue.

Experiment. Place a little in water. It does not dissolve, but dissolves readily when a drop of sodium carbonate solution is added.

Experiment. Repeat the Murexid test with some of the sediment.

Experiment. Repeat Schiff's test with the solution of the sediment obtained above. A black reduction is the result.

CALCIUM OXALATE, CaC_2O_4.

Heat some on the foil. It blackens slightly but does not burn or disappear. Place this residue in a watch glass and add a little water and a drop of acetic acid. It effervesces, due to the discharge of carbon dioxide, and dissolves. Retain this solution.

The application of heat to the sediment has converted the calcium oxalate into calcium carbonate, which effervesces with an acid. $CaC_2O_4 = CaCO_3 + CO$.

Experiment. Heat another portion of the sediment for a few minutes in the flame. Place a particle on a piece of moistened red litmus paper. It turns it blue, showing that calcium oxide has been formed by the protracted heating of the calcium oxalate. $CaC_2O_4 = CaO + CO + CO_2$.

Place the remainder of the residue in some water in a test tube or watch glass. It dissolves, especially on the addition of a drop of acetic acid.

Experiment. Add some ammonium oxalate to some of the solution obtained above. A white precipitate of calcium oxalate shows the presence of calcium.

Calcium oxalate does not give the Murexid test.

ACID CALCIUM PHOSPHATE, $Ca(H_2PO_4)_2$.

Experiment. Heat some on the foil. It does not blacken nor does it burn up. No apparent change.

Experiment. Place some in a test tube with water. Add some acetic acid and apply heat if necessary. It

dissolves. Divide the solution in two portions. To one portion add a solution of ammonium oxalate. A white precipitate of calcium oxalate shows the presence of calcium.

To the other portion add a solution of uranium acetate. A white precipitate of uranium phosphate denotes the presence of phosphoric acid.

Acid calcium phosphate does not give the Murexid test.

Di-calcium phosphate, $CaHPO_4$.

Tri-calcium phosphate, $Ca_3(PO_4)_2$.

Occurring in neutral or alkaline urines are tested for as described under acid calcium phosphate. Their microscopic appearances are different.

CALCIUM CARBONATE, $CaCO_3$.

Experiment. Heat some on the foil. It does not blacken nor burn up. No apparent change. Place a particle of the heated residue on a piece of moistened red litmus paper. It turns blue, showing the presence of calcium oxide, formed by heating the carbonate. $CaCO_3 = CaO + CO_2$.

Experiment. Place some of the sediment in a test tube with water. It does not dissolve, but readily dissolves on the addition of a drop or two of acetic acid with an effervescence showing that it is a carbonate by the escape of carbon dioxide gas.

Experiment. Add some ammonium oxalate to the solution obtained in the previous experiment. A white precipitate of calcium oxalate shows the presence of calcium.

This sediment does not give the Murexid test.

AMMONIUM-MAGNESIUM PHOSPHATE OR TRIPLE PHOSPHATE, NH_4, MG, PO_4.

Heat on the piece of foil. It does not blacken, but gives off an odor of ammonia, which gives dense white fumes with a drop of hydrochloric acid on a glass rod.

A residue of magnesium pyro-phosphate is left on the foil.

Experiment. Place some of the sediment in a test tube and add some caustic soda. A marked odor of ammonia is evolved, and a piece of moistened red litmus paper is turned blue by the vapors, if held at the mouth of the test tube.

Experiment. Place some of the sediment with water in a test tube and add a few drops acetic acid. It dissolves without effervescence.

Experiment. Add some uranium acetate to some of the above solution. A white precipitate of uranium phosphate denotes the presence of phosphoric acid.

It does not respond to the Murexid test.

AMMONIUM URATE, $(N_2H_4)C_5H_2N_4O_3$.

Experiment. Heat some on the foil. It blackens and burns up, leaving no residue.

Experiment. Place some of the sediment on a watch glass and add a few drops caustic soda. Apply a gentle heat to the contents of the watch glass, and hold a piece of moistened red litmus directly over the watch glass. It turns blue, showing that ammonia has been evolved by the action of the caustic soda on the ammonium urate.

Experiment. Place some of the sediment in water in

a test tube. It does not dissolve, but readily dissolves on the addition of a few drops sodium carbonate.

Experiment. Apply Schiff's test to the above solution. A black reduction shows the presence of uric acid.

Experiment. Apply the Murexid test to some of the sediment on a watch glass. A purple coloration denotes the presence of uric acid.

Cystin, leucin, tyrosin, and xanthin have been observed in sediments in very rare cases.

CALCULI.

Calculi may consist of only one ingredient, but more frequently two or more deposits occur in separate layers. Most calculi have a nucleus generally of uric acid.

In examining a calculus, it should be wrapped up in a piece of writing paper and cut through the centre by means of a fine saw. Each layer should be examined separately by the following scheme:

Does not burn.		Does burn.		
The powder when treated with H Cl.		With flame.	Without flame.	
Does not effervesce.			The powder gives the Murexid test.	No noticeable ammonia reaction.	Uric acid.
			The powder, when treated with N a O H, gives	Strong ammonia reaction.	Ammonium urate.
The gently heated powder with H Cl.				Does not give the Murexid test. The powder dissolves in HNO_3 without effervescence. The dried yellow residue becomes orange with alkali, beautiful red on warming.	Xanthin.
		Flame pale blue, burns a short time. Peculiar sharp odor. The powder dissolves in ammonia and six-sided plates separate on the spontaneous evaporation of the ammonia.			Cystin.
		Flame yellow, pale, continuous. Odor of resin or shellac on burning. Powder soluble in alcohol and ether.			Urostealith.
		Flame yellow, continuous. Odor of burnt feathers. Insoluble in alcohol and ether. Soluble in N a O H with heat. Precipitated herefrom by acetic acid and generation of H_2S.			Fibrin.
Effervesces.					Calcium carbonate.
The powder when moistened with a little N a O H.	Effervesces.				
	No, NH_3 or at least only traces of NH_3. Powder dissolves in acetic acid or H Cl. This solution is precipitated by ammonia (amorphous).				Bone earth (phosphate of calcium and magnesium).
	Abundant ammonia. The powder dissolves in acetic acid or H Cl. This solution gives a crystalline precipitate with ammonia.				Triple phosphate mixed with unknown amount of earthy phosphate.

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